Jpn. J. Pharmacol. **89**, 216 – 220 (2002)

FORUM MINIREVIEW

Recent Advances in the Search for the μ-Opioidergic System

The Antinociceptive Properties of Endomorphin-1 and Endomorphin-2 in the Mouse

Leon F. Tseng*

Department of Anesthesiology, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226, USA

Received December 4, 2001 Accepted March 22, 2002

ABSTRACT—Two highly selective μ-opioid receptor agonists, endomorphin-1 (EM-1) and endomorphin-2 (EM-2), have been identified and postulated to be endogenous μ-opioid receptor ligands. The present minireview describes the antinociceptive properties with the tail-flick test of these two ligands given intracerebroventricularly (i.c.v.) and intrathecally (i.t.) in ICR mice. EM-1 or EM-2 given i.c.v. or i.t. dose-dependently produce antinociception. These antinociceptive effects induced by EM-1 and EM-2 given i.c.v. or i.t. are selectively mediated by the stimulation of μ-, but not δ- or κ-opioid receptors. Like other μ-opioid agonists morphine and DAMGO ([D-Ala^2,*N*MePhe^4,Gly^5-ol]enkephalin), EM-1 and EM-2 given i.c.v. activate descending pain controls by the releases of noradrenaline and 5-HT and subsequently act on α_2-adrenoceptors and 5-HT receptors, respectively, in the spinal cord to produce antinociception. However, the antinociception induced by EM-2 given i.c.v. or i.t. also contain an additional component, which is mediated by the release of dynorphin A(1 – 17) acting on κ-opioid receptors at the supraspinal and spinal sites. In addition, the antinociception induced by EM-2 given i.c.v. contains another component, which is mediated by the release of Met-enkephalin acting on δ_2-opioid receptors in the spinal cord. It is proposed that there are two subtypes of μ-opioid receptors,which are involved in EM-1- and EM-2-induced antinociception. One subtype of μ-opioid receptors is stimulated by EM-1, EM-2 and other μ-opioid agonists morphine and DAMGO; and another subtype of μ-opioid is sorely stimulated by EM-2 and is involved in the releases of dynorphin A(1 – 17) and Met-enkephalin for the production of antinociception.

Keywords: Endomorphin-1, Endomorphin-2, Antinociception, μ-Opioid receptor, Descending pain control system

Since the initial demonstration of μ-opioid receptors (MOP-Rs) over 25 years ago, investigators have searched for their endogenous ligands. The search led to the discovery of enkephalins, endorphins and dynorphins in the 1970's, yet they have either low selectivity or efficacy at the MOP-Rs. The enkephalins are the endogenous ligands for δ-opioid receptors (DOP-Rs) and dynorphin A(1 – 17) is the endogenous ligand for κ-opioid receptors (KOP-Rs). β-Endorphin has been proposed to be an endogenous ligand for the ε-opioid receptor (1, 2). However, it also binds equally well to MOP- and DOP-Rs with high affinity. Thus, many investigators felt that these peptides were not the endogenous ligands for MOP-Rs because of their selectivity profiles.

*Corresponding author. FAX: +1-414-456-6507
E-mail: ltseng@mcw.edu

Recently, two new peptides, endomorphin-1 (EM-1) and endomorphin-2 (EM-2), have been isolated from mammalian brain and found to activate MOP-Rs with high affinity and selectivity, raising the possibility that they are two endogenous MOP-R ligands (3). In opioid receptor binding assays, both EM-1 and EM-2 compete with μ_1 (MOP1)- and MOP2-R sites potently (4). Neither compound has appreciable affinities for DOP-Rs and KOP1-Rs. EMs were found in the brain and spinal cord regions which are also rich in MOP-R (3, 5 – 9). Intrathecal (i.t.) or intracerebroventricular (i.c.v.) injection of EMs produces potent analgesia, which is blocked by the pretreatment with the MOP-R antagonists, naloxone or β-funaltrexamine (β-FNA) (4, 9 – 11). In MOP-R deficient CXBK mice or MOP-R knockout mice, neither EM-1 nor EM-2 produces any inhibition of the tail-flick and hot-plate responses, indicating that MOP-Rs play an essential role in mediat-

ing EM-induced antinociception (4, 12, 13). The present miniview describes in detail the antinociceptive properties of the EM-1 and EM-2 given supraspinally or spinally in the mouse.

Differential antinociception induced by EM-1 and EM-2 given i.c.v. in the mouse

Intracerebroventricular injection of EM-1 or EM-2 inhibits dose-dependently the tail-flick response. These antinociceptive effects induced by EM-1 or EM-2 reach their peaks 5 min after injection, rapidly decline and return to the preinjection level 20 min after injection. The duration of the tail-flick inhibition induced by EM-1 appears to be longer than that of EM-2. Also, the ED_{50} value for EM-2 in inhibiting the tail-flick response is about 3.3-fold higher than that of EM-2 (Table 1). The slope of the dose-response curve of EM-2 for inhibition of the tail-flick response was significantly steeper than that of EM-1. The different slope functions of the dose-response lines for EM-1 and EM-2 for inhibition of the tail-flick response suggest that these two peptides may produce antinociception by different modes of action (14).

The original description of EMs reveals that both compounds have a profound μ selectivity (3). Both EMs compete for μ binding sites over 1000-fold more effectively than for either DOP-Rs or KOP1-Rs (3). Goldberg et al. (4) also confirm that both EM-1 and EM-2 compete for both MOP1-R and MOP2-R sites quite potently, but have no appreciable affinity for either DOP-Rs or KOP1-Rs. The inhibition of the tail-flick and hot-plate responses induced by either EM-1 or EM-2 given i.c.v. is blocked completely by the selective μ-opioid receptor antagonist β-FNA, but not by the DOP1-R antagonist 7-benzylidenenaltrexone or the DOP2-R antagonist naltriben. The findings are consistent with the view that these two endomorphins are selective MOP-R ligands and the antinociception induced by EM-1 and EM-2 is mediated by the selective stimulation of MOP-Rs, but not by DOP1-Rs or DOP2-Rs.

However, the antinociception induced by EM-2, but not EM-1, is also partially blocked by the pretreatment with the KOP-R antagonist nor-binaltorphimine (nor-BNI), indicating that the antinociception induced by EM-2, but not EM-1, produces its antinociception in part by the stimulation of KOP-Rs. Because EM-2 has a very low affinity for KOP-Rs in in vitro ligand-binding assays, it is unlikely that the EM-2-induced antinociception is mediated by the direct stimulation of KOP-Rs. It is most likely that EM-2 produces its antinociception by the release of dynorphin A(1 – 17), which subsequently acts on KOP-Rs. This is evidenced by the finding that the pretreatment of mice with an antiserum against dynorphin A(1 – 17), which binds the released dynorphin A(1 – 17) attenuates the antinociception induced by EM-2. However, the pretreatment with nor-BNI or antiserum against dynorphin A(1 – 17) even at high doses only partially but not completely blocked the antinociception induced by EM-2, suggesting that EM-2-induced antinociception is only mediated in part by a κ-minergic mechanism.

Table 1. The ED_{50} values for endomorphin-1 and endomorphin-2 given i.c.v. or i.t. to produce tail-flick inhibition in the mouse

	ED_{50} (95% confidence limits) nmol	Potency ratio
i.c.v. administration		
Endomorphin-1	6.16 (4.42 – 8.57)	
Endomorphin-2	20.27 (16.07 – 25.57)	3.29
i.t. administration		
Endomorphin-1	2.80 (2.28 – 3.46)	
Endomorphin-2	6.26 (5.18 – 7.57)	2.24

Differential mechanisms mediating descending pain controls for antinociception induced by supraspinally-administered EM-1 and EM-2 in the mouse

The activation of spinipetal descending pain controls systems by OP-R agonists plays a major role in antinociception induced by stimulation of various opioid agonists given supraspinally. These antinociceptive effects induced by the stimulation of various opioid agonists given supraspinally involve multiple descending pain control pathways. The antinociception induced by MOP-R agonists such as morphine and DAMGO ([D-Ala^2,*N*MePhe^4,Gly^5-ol]enkephalin) given supraspinally is mediated by the release of noradrenaline and 5-HT acting on α_2-adrenoceptors and 5-HT receptors, respectively, in the spinal cord (15, 16), whereas the antinociception induced by KOP-R agonists such a U50,488H and bremazocine given supraspinally is mediated by the release of dynorphin A(1 – 17) acting on KOP-Rs (17). The antinociception induced by β-endorphin given supraspinally is mediated by the release of Met-enkephalin acting on DOP2-Rs (1, 2).

Inasmuch as the antinociception induced by either EM-1 or EM-2 given supraspinally is mediated by the stimulation of MOP-Rs (14), it is anticipated that both EM-1 and EM-2 given supraspinally will also utilize the same descending pain control pathways as that of other μ-opioid agonists such as morphine and DAMGO for producing antinociception. Indeed, the inhibition of α_2-adrenoceptors and 5-HT receptors by i.t. treatment with yohimbine and methysergide, respectively effectively inhibited the antinociception induced by i.c.v.-administered EM-1 and EM-2. Thus, like morphine and DAMGO, EM-1 and EM-2 activate the spinipetal noradrenergic and serotonergic systems and releases of noradrenaline and 5-HT acting on α_2-adrenoceptors and 5-HT-receptors, respectively, in the

spinal cord for producing antinociception (18).

In addition to the monoaminergic descending pain control systems, which are activated by EM-1 and EM-2, two additional opioidergic descending pathways are also involved in antinociception induced by supraspinally-administered EM-2, but not by EM-1. This is evidenced by the finding that i.t. pretreatment with the DOP2-R antagonist naltriben or the KOP-R antagonist nor-BNI attenuates the antinociception produced by i.c.v.-administered EM-2. The effect appears to be due to the specific inhibition of DOP2-R and KOP-R, because i.t. pretreatment with the MOP-R antagonist CTOP (D-Phe-Cys-Tyr-D-Trp-Orm-Thr-Pen-Thr-NH_2) or the DOP1-R antagonist 7-benzylidenenaltrexone does not inhibit antinociception induced by EM-2. Since the DOP2-R and KOP-R are the receptors for endogenous ligands Met-enkephalin and dynorphins, respectively, it is then expected that the effects are mediated by the release of Met-enkephalin and dynorphin A(1 – 17). Indeed, i.t. pretreatment with an antiserum against Met-enkephalin or dynorphin A(1 – 17) significantly attenuates the antinociception induced by EM-2. On the other hand, i.t. pretreatment with antiserum against β-endorphin or Leu-enkephalin does not affect the antinociception induced by i.c.v.-administered EM-2. Thus, antinociception induced by supraspinally-administered EM-2 contains other additional components, which are mediated by the releases of Met-enkephalin and dynorphin A(1 – 17) acting on DOP2-R and KOP-R, respectively, in the spinal cord (18).

Differential antinociception induced by spinally-administered EM-1 and EM-2 in the mouse

Intrathecal injection of EM-1 or EM-2 dose-dependently causes an increase of the inhibition of the tail-flick response. The inhibition reached its peak 5-min after injection, rapidly declined and returned to the preinjection level 20 min after injection. The duration of the antinociception induced by EM-1 and EM-2 given spinally is about the same, but EM-1 is about twofold more potent than that of EM-2. The ED_{50} values for EM-1 and EM-2 given i.t. for the inhibition of the tail-flick inhibition are shown in Table 1.

The antinociception induced by either EM-1 or EM-2 injected i.t. is completely blocked by i.t. pretreatment with CTOP, indicating that the spinally-administered EM-1- and EM-2-induced antinociception is also mediated by the stimulation of MOP-Rs in the spinal cord (19). Earlier, Stone et al. (11) reported that antinociception induced by EM-1 and EM-2 given i.t. is blocked by i.t.-pretreated naloxone in the tail-flick test. The importance of MOP-Rs for EM-1 and EM-2 to produce antinociception is also supported by our previous studies. Both EM-1 and EM-2 do not activate G-proteins in the spinal cord and pons/medulla tissues of the MOP-R knockout mice (13, 20) and both peptides given i.c.v. fail to produce any antinociception in MPO-R knockout mice (13).

However, the antinociception induced by i.t.-administered EM-2, but not EM-1, contains an additional component, which is mediated by the stimulation of KOP-Rs in the spinal cord. This view is supported by the finding that antinociception induced by EM-2, but not EM-1 given i.t. is attenuated by i.t. pretreatment with the KOP-R antagonist nor-BNI, indicating the involvement of KOP-Rs in the spinal cord for EM-2-induced antinociception (19). However, nor-BNI even at high doses that completely blocks the selective KOP-R agonist-induced antinociception (21) only partially, but not entirely, blocked the antinociception induced by EM-2. The finding appears to indicate that the EM-2-induced antinociception is mediated only in part by a KOP-R mechanism in the spinal cord (19). Since EM-2 has a very low affinity for KOP-Rs in the in vitro receptor binding assay (3), it is unlikely that this EM-2-induced antinociception is due to a direct stimulation of KOP-Rs by EM-2. This view is further supported by our previous finding that EM-2 does not activate G-proteins with [^{35}S]GTPγS binding in the spinal cord tissue obtained from MOP-R knockout mouse, which is still responsive to the KOP-R agonist for G-protein activation (22).

This κ-minergic mechanism for EM-2-induced antinociception is mediated by the release of dynorphin A(1 – 17). This view is supported by the finding that i.t. pretreatment with an antiserum against dynorphin A(1 – 17) attenuates the antinociception induced by i.t.-injected EM-2, but not EM-1. Thus activation of MOP-Rs by EM-2 initially induces the release of dynorphin A(1 – 17), which subsequently acts on KOP-Rs for the production of antinociception. We propose that there are two subtypes of MOP-Rs which are involved in EM-1- and EM-2-induced antinociception. One subtype of MOP-Rs is stimulated by both EM-1 and EM-2 and another subtype of MOP is solely stimulated by EM-2 and is involved in the release of dynorphin A(1 – 17) acting on KOP-Rs for the production of antinociception.

Unlike the antinociception induced by EM-2 given i.c.v., which is also mediated by the release of Met-enkephalin acting on DOP2-Rs in the spinal cord, EM-2 given spinally does not cause any release of Met-enkephalin to produce DOP-R-mediated antinociception. This view is supported by the finding that i.t. pretreatment with antiserum against Met-enkephalin or DOP2-R antagonist naltriben fails to affect the tail-flick inhibition induced by i.t.-administered EM-2 (19). However, another study using the thermal paw-withdrawal test demonstrated that i.t. pretreatment with antiserum against Met-enkephalin attenuates the paw-withdrawal inhibition induced by i.t.-administered EM-2, indicating that i.t. administration of EM-2 may release Met-

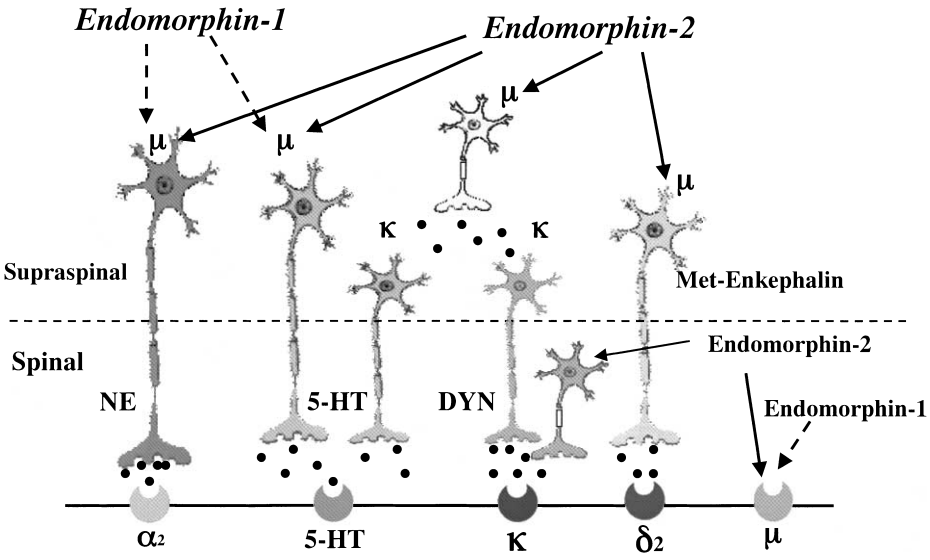

Fig. 1. Simplified diagram depicting different spinal and descending pain modulatory pathways activated by endomorphin-1 and endomorphin-2. Two different subtypes of μ-opioid receptors are proposed for endomorphin-1 and endomorphin-2 to produce antinociception. One subtype of μ-opioid receptors is stimulated by both endomorphin-1 and endomorphin-2, and the antinociception induced by endomorphin-1 or endomorphin-2 given supraspinally is mediated by the releases of noradrenaline (NE) and 5-HT acting on α_2-adrenoceptors and 5-HT receptors, respectively (left). Another subtype of μ-opioid receptors is solely stimulated by endomorphin-2 and the antinociception induced by endomorphin-2 given supraspinally is mediated by the releases of dynorphin A(1 – 17) (DYN) acting on κ-opioid receptors at the supraspianl and spinal sites and Met-enkephalin acting on δ_2-opioid receptors in the spinal cord. Endomorphin-2 given spinally releases dynorphin A(1 – 17) acting on κ-opioid receptors to produce antinociception (14, 18, 19).

enkephalin from the spinal cord (23).

Others from different laboratories also reported the different antinociceptive effects induced by EM-1 and EM-2. Systemic pretreatment with the MOP1-R antagonist naloxonazine attenuates the antinociception induced by EM-2, but not EM-1 given i.t. or i.c.v., suggesting that antinociception induced by EM-2, but not EM-1 is mediated by the stimulation of naloxonazine sensitive MOP-Rs (24, 25). Pretreatment with different antisense oligodeoxynucleotides (ODN) against a different G-protein subunit is also be able to differentiate antinociceptive effects induced by EM-1 and EM-2. Intrathecal pretreatment with antisense ODN against G-protein subunit Giα_2 protein attenuates the antinociception induced by i.t.-administered EM-2, but not EM-1, while i.t. pretreatment with antisense ODN against G-protein subunits of Giα_1, Giα_3 or Gzα does not affect the antinociception induced by either EM-1 or EM-2 (26). It is most likely that the differential antinociceptive effects observed are mediated by stimulation of different subtypes of MOP-Rs.

Conclusion

It is concluded that both EM-1 and EM-2 given supraspinally or spinally produces their antinociception by the stimulation of MOP-Rs. Like other MOP-R agonists morphine or DAMGO, EM-1 and EM-2 given supraspinally activate descending pain controls by the releases of noradrenaline and 5-HT acting on α_2-adrenoceptors and 5-HT receptors, respectively, in the spinal cord to produce antinociception. However, the antinociception induced by EM-2 given supraspinally contain additional components, which are mediated by the release of dynorphin A(1 – 17) acting on KOP-Rs at the supraspinal and spinal sites and the release of Met-enkephalin acting on DOP2-Rs in the spinal cord. The antinociception induced by EM-2 given i.t. also contains an additional component, which is mediated by the release of dynorphin A(1 – 17) acting on KOP-Rs in the spinal cord. It is most likely that different subtypes of MOP-Rs are involved in EM-1 and EM-2 antinociception (Fig. 1).

Acknowledgments

This work was supported by grant DA03811 from the National Institute of Health, National Institute on Drug Abuse, USA.

REFERENCES

1 Narita M and Tseng LF: Evidence for the existence of β-endorphin-sensitive "ε-opioid receptor" in the brain: the mechanisms of ε-mediated antinociception. Jpn J Pharmacol **76**, 233 – 253 (1998)

2 Tseng LF: Mechanisms of β-endorphin-induced antinociception. *In* The Pharmacology of Opioid Peptides, Edited by Tseng LF, pp 249 – 269, Harwood Academic Publishers GMBH, Chur, Switzerland (1995)

3 Zadina JE, Hackler L, Ge L-J and Kastin AJ: A potent and selective endogenous agonist for the μ-opiate receptor. Nature **386**, 499 – 502 (1997)

4 Goldberg IE, Rosi GC, Letchworth SR, Mathis JP, Ryan-Moro J, Leventhal L, Su W, Emmel D, Bolan EA and Pasternak GW: Pharmacological characterization of endomorphin-1 and endomorphin-2 in mouse brain. J Pharmacol Exp Ther **286**, 1007 – 1013 (1998)

5 Martin-Schild S, Zadina JE, Gerall AA, Vigh S and Kastin AJ: Localization of endomorphin-2-like immunoreactivity in the rat medulla and spinal cord. Peptides **18**, 1641 – 1649 (1997)

6 Anton B, Martinez I, Acevedo R, Calva JC, Valdez A, Leon-Olea A and Leff P: Immunohistochemistry for endomorphins in the rat CNS. Soc Neurosci Abstracts **24**, 851 (1998)

7 Pierce TL, Grahek MD and Wessendorf MW: Immunoreactivity for endomorphin-2 occurs in primary afferents in rats and monkey. NeuroReport **9**, 385 – 389 (1998)

8 Pierce TL and Wessendorf MW: Distribution of endomorphin-2 immunoreactivity in rat brain. Soc Neurosci Abstracts **24**, 851 (1998)

9 Schreff M, Schulz S, Wiborny D and Hollt V: Immunofluorescent identification of endomorphin-2-containing nerve fibers and terminals in the rat brain and spinal cord. NeuroReport **9**, 1031 – 1034 (1998)

10 Bodnar RJ, Krzanowska E, Leventhal L, Rossi GC, Mathis JP and Pasternak GW: Pharmacological characterization of endomorphin-I and endomorphin-II analgesia and gastrointestinal transit in mice. Soc Neurosci, Abstract **23**, 680 (1997)

11 Stone LS, Fairbanks CA, Laughlin TM, Nguyen HO, Bushy TM, Wessendorf MW and Wilcox GL: Spinal analgesic actions of the new endogenous opioid peptides endomorphin-1 and -2. NeuroReport **8**, 3131 – 3135 (1997)

12 Tseng LF, Suganuma C, Narita M, Oji GS, Tseng EL, Bhaita A, Mizoguchi H and Nagase H: Differential mechanism mediating antinociception induced by supraspinally administered newly discovered endogenous opioid peptides endomorphin-1 and endomorphin-2 in the mouse. Soc Neurosci Abstr **24**, 1356 (1998)

13 Mizoguchi H, Narita M, Oji DE, Suganuma C, Nagase H, Sora I, Uhl GR, Cheng EY and Tseng LF: μ-Opioid receptor genedose dependent reductions in G-protein activation in the pons /medulla and antinociception induced by endomorphins in μ-opioid receptor knockout mice. Neuroscience **94**, 203 – 207 (1999)

14 Tseng LF, Narita M, Suganuma C, Mizoguchi H, Ohsawa M, Nagase H and Kampine JP: Differential antinociceptive effects of endomorphin-1 and endomorphin-2 in the mouse. J Pharmacol Exp Ther **292**, 576 – 583 (2000)

15 Tseng LF and Tang R: Differential mechanisms mediate β-endorphin- and morphine-induced inhibition of the tail-flick response in rats. J Pharmacol Exp Ther **252**, 546 – 551 (1990)

16 Tseng LF and Collins KA: Different mechanisms mediating tail-flick inhibition induced by β-endorphin, DAMGO and morphine form Rob and GiA in anesthetized rat. J Pharmacol Exp Ther **257**, 530 – 538 (1991)

17 Tseng LF and Collins KA: Spinal involvement of both dynorphin A and Met-enkephalin in the antinociception induced by intracerebroventricularly administered bremazocine but not morphine in the mouse. J Pharmacol Exp Ther **266**, 1430 – 1438 (1993)

18 Ohsawa M, Mizoguchi H, Narita M, Chu M, Nagase H and Tseng LF: Differential mechanisms mediating descending pain controls for antinociception induced by supraspinally-administered endomorphin-1 and endomorphin-2 in the mouse. J Pharmacol Exp Ther **294**, 1106 – 1111 (2000)

19 Ohsawa M, Mozoguchi H, Narita M, Nagase H, Kampine JP, and Tseng LF: Differential antinociception induced by spinally administered endomorphin-1 and endomorphin-2 in the mouse. J Pharmacol Exp Ther **298**, 592 – 597 (2001)

20 Narita M, Mizoguchi H, Oji GE, Tseng EL, Suganuma C, Nagase H and Tseng LF: Characterization of endomorphin-1 and -2 on [^{35}S]GTPγS binding in the mouse spinal cord. Eur J Phamacol **351**, 383 – 387 (1998)

21 Tseng LF, Wang HQ and Xu JY: Inhibition of spinal nitoric oxide synthase by N^{ω}-nitro-L-arginine blocks the release of Met-enkephalin and antinociception induced by supraspinally administered β-endorphin in the rat. Neuroscience **78**, 461 – 467 (1997)

22 Narita H, Mizoguchi H, Narita M, Sora I, Uhl GR and Tseng LF: Absence of G-protein activation by endomorphin-1 and -2 in the spinal cord of μ-opioid receptor knockout mice. Br J Pharmacol **126**, 451 – 456 (1999)

23 Sakurada S, Hayashi T, Yuki M, Orito T, Zadina JE, Kastin A, Fujimura T, Murayama K, Sakurada C, Sakurada T, Narita M, Suzuki T, Tan-no K and Tseng LF: Differential antinociceptive effects induced by intrathecally administered endomorphin-1 and endomorphin-2 in the mouse. Eur J Pharmacol **427**, 203 – 210 (2001)

24 Sakudara S, Zadina JE, Kastin AJ, Katsuyama S, Fujimura T, Murayama K, Yuki M, Ueda H and Sakurada T: Differential involvement of μ-opioid receptor subtypes in endomorphin-1- and endomorphin-2-induced antinocicepion. Eur J Pharmacol **372**, 25 – 30 (1999)

25 Sakurada S, Hayashi T, Yuhki M, Fujimura T, Maruyama K, Yonezawa A, Sakurada C, Takeshita M, Zadina JE, Kastin AJ and Sakurada T: Differential antagonism of endomorphin-1 and endomorphin-2 spinal antinociception by naloxonazine and 3-methoxynaltrexone. Brain Res **881**, 1 – 8 (2000)

26 Sánchez-Blázquez P, Rodríguez-Díaz M, Deantonio I and Garzón J: Endomorphin-1 and endomorphin-2 show differences in their activation of μ opioid receptor regulated G-proteins in supraspinal antinociception in mice. J Pharmacol Exp Ther **291**, 12 – 18 (1999)